Exhibit 3

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Short-term ozone exposure and asthma severity: Weight-of-evidence analysis

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ABSTRACT

To determine whether evidence indicates that short-term exposure to ambient concentrations of ozone in the United States can affect asthma severity, we systematically reviewed published controlled human exposure, epidemiology, and animal toxicity studies. The strongest evidence for a potential causal relationship came from epidemiology studies reporting increased emergency department visits and hospital admissions for asthma following elevated ambient ozone concentrations. However, while controlled exposure studies reported lung function decrements and increased asthma symptoms following high ozone exposures 160–400 parts per billion [ppb]), epidemiology studies evaluating similar outcomes reported less consistent results. Animal studies showed changes in pulmonary function at high ozone concentrations (> 500 ppb), although there is substantial uncertainty regarding the relevance of these animal models to human asthma. Taken together, the weight of evidence indicates that there is at least an *equal* likelihood that either explanation is true, *i.e.*, the strength of the evidence for a causal relationship between short-term exposure to ambient ozone concentrations and asthma severity is "***equipoise and above***."

1. Introduction

Ozone, a colorless gas with a distinctively pungent smell, is naturally present in the upper atmosphere. In the presence of sunlight, ozone is also generated at ground level from photochemical reactions between precursor pollutants, including volatile organic compounds (VOCs), oxides of nitrogen (NO_x), and carbon monoxide (CO) (US EPA, 2013). Ozone is a powerful oxidizing agent and, at high concentrations, can harm living organisms and materials. People are exposed to ground-level ozone both indoors and outdoors as they participate in normal daily activities (US EPA, 2013). Ambient ozone concentrations are routinely monitored in the US, and the median daily average, 8-h maximum, and 1-h maximum ozone concentrations across all US sites between 2007 and 2009 were 29, 40, and 44 parts per billion (ppb), respectively (US EPA, 2013). The 99th percentiles of these ozone concentration metrics are 60, 80, and 94 ppb, respectively (US EPA, 2013). Ground-level ozone is one of the six criteria air pollutants regulated by the United States Environmental Protection Agency (US EPA). The current National Ambient Air Quality Standard (NAAQS) for ozone is 70 ppb for the annual fourth-highest daily maximum 8-h concentration, averaged over three years.

Asthma is a multifactorial, heterogeneous disease involving chronic airway inflammation, variable airflow obstruction, and airway hyperresponsiveness (AHR) to various triggers (Currie and Baker, 2012; Grainge and Davies, 2013; Myers and Tomasio, 2011). It is a relatively common disease, with an estimated prevalence in the US between 2008 and 2010 of 9.5% in children 0–17 years old and 7.7% in adults (Moorman et al., 2012). Asthma etiology is complex, and a specific cause has yet to be identified. Genetics and respiratory infections are the most well-established risk factors (Myers and Tomasio, 2011). A diagnosis of asthma is typically based on lung function tests showing reduced expiratory flow rate, reactivity to bronchoconstrictors such as methacholine, and response to bronchodilators such as albuterol (Mayo Clinic, 2014a). Common pathological features of the airways include epithelial hyperplasia, increased smooth muscle mass, fibrotic thickening of the subepithelial basement membrane, and decreased antioxidant capacity (Barnes, 2008; Currie and Baker, 2012; Grainge and Davies, 2013).

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Asthma exacerbations (*i.e.*, asthma attacks), which involve reversible narrowing of the airways, and symptoms such as wheezing, shortness of breath, and chest tightness or pain, are hypothesized to occur when an acute inflammatory response is added to the underlying chronic airway inflammation (Barnes, 2008; Moorman et al., 2012). Although inflammation appears to play a role, how inflammatory cells interact and how this interaction translates into asthma symptoms that might constitute an asthma exacerbation requiring intervention is uncertain (Reddel et al., 2009; Barnes, 2008). While several cell types have been implicated in severe asthma (eosinophils, neutrophils, and granulocytes), the number of these cells in sputum varies widely across patients and even intraindividually on a monthly basis (Chung et al., 2014). Common triggers for asthma exacerbations include allergens, viral respiratory infections, exercise, tobacco smoke, cold air, gastroesophageal reflux disease, and stress (Barnes, 2008; Sears, 2008; Mayo Clinic, 2014b). In addition, several air pollutants, including ozone, nitrogen dioxide (NO_2), and particulate matter (PM) have been hypothesized to trigger asthma exacerbations (Barnes, 2008a, 2008b; Guarnieri and Balmes, 2014; Sears, 2008).

Numerous observational and experimental (*i.e.*, controlled exposure and animal toxicity) studies have investigated whether short-term ozone exposures may affect asthma severity (US EPA, 2013). Epidemiology studies have assessed this relationship by evaluating respiratory symptoms, medication use, and changes in lung function among people with asthma. Severe asthma exacerbations manifest as visits to primary care doctors or emergency departments (ED), or as hospital admissions (HA), which have also been evaluated extensively in observational studies. In experimental settings, people with asthma have been exposed to specific ozone concentrations in a controlled environment, and their responses (usually changes in lung function parameters) have been measured. Laboratory studies have also been conducted in different animal models for asthma, although their relevance to humans is unclear (*e.g.*, Hatch et al., 2013).

In its most recent review of the ozone NAAQS, US EPA concluded that short-term ozone exposure causes respiratory morbidity, and that individuals with asthma constitute a group susceptible to ozone. US EPA based these conclusions largely on observed associations between short-term ozone exposure and various outcomes related to asthma severity, including ED visits and HA for asthma, as well as changes in lung function and reported respiratory symptoms in individuals with asthma (US EPA, 2013).

We conducted detailed systematic reviews of the epidemiology and controlled human exposure studies that focused on ozone exposure and asthma severity on this issue (See Supplemental materials). We also systematically reviewed animal toxicity studies that evaluated the effects of ozone exposures in laboratory animal asthma models (See Supplemental materials). Herein, we integrate evidence across disciplines to determine whether the weight of evidence (WoE) indicates that short-term exposure to ambient ozone concentrations can impact asthma severity, as reflected by measures of lung function, symptoms, and frequency of exacerbation events.

2. Methods

We addressed the question: Does short-term exposure to ozone at ambient concentrations affect asthma severity? We defined short-term as fewer than 30 days, based on criteria established by US EPA (2013). Although our research question is focused on ambient exposure concentrations, we evaluated studies of any concentration of ozone, to enable an evaluation of overall hazard as well as a biological gradient. We relied on asthma severity endpoints as defined by recent American Thoracic Society (ATS) guidance, including symptoms, hospitalization, medication use, and lung function (Reddel et al., 2009). As discussed in the Supplemental materials, because inflammatory endpoints are variable and their relevance is unclear, we did not review studies of inflammatory cells or markers.

In WoE analyses, evaluating study quality is critical because results from studies with more robust designs and methodology should carry more weight in evidence integration. We developed distinct study quality criteria for each realm of evidence (*i.e.*, controlled human exposure, epidemiology, and laboratory animal studies). The study quality criteria were based on those used in previous study quality evaluations (*e.g.*, Goodman et al., 2014; Prueitt et al., 2014), and the criteria were informed by several existing guidelines and quality evaluation systems, including the National Toxicology Program (NTP) Office of Health Translation (OHAT) risk-of-bias (RoB) tool, the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guidelines (Kilkenny et al., 2010), and other international research guidelines, such as those of the Organization for Economic Co-operation and Development (OECD) and World Health Organization (WHO) (OECD, 1998). The specific criteria for each realm of evidence are described in more detail in the Supplemental materials.

We relied on these criteria to evaluate the quality of individual studies and to categorize them as being of higher or lower quality. We used a scoring system in which we assigned each study a score of − 1 or + 1 for each criterion. These scores are intended to be only a crude measure of quality and are not intended to be summed for a ranking of studies, since we did not assign any weight to each criterion. Instead, based on the quality scores, we simply grouped the studies into two tiers: Tier I indicates a study with a greater number of strengths than limitations (the number of positive attributes outweighed the number of negative attributes), and Tier II indicates a study with a greater number of limitations than strengths (the positives did not outweigh the negatives). Because even one particular strength or limitation could "outweigh" all the others in terms of its impact on the interpretation of results, we only used this system to divide the studies into two groups. We also evaluated all of the study quality criteria for each individual study and addressed additional factors not included in our scoring system that may affect the interpretation of individual study results (discussed below). We evaluated all individual studies in both tiers but gave Tier I studies more weight in the analysis, because Tier II studies are of lower quality.

We integrated the evidence from controlled human exposure studies, epidemiology studies, and animal toxicity studies (Described in Supplemental materials). We integrated the evidence across these realms in the context of several of the Bradford Hill aspects, including strength of association, consistency of associations, coherence, biological gradient, biological plausibility, and temporality, as well as confounding, bias, and the clinical relevance of effects. We did this to determine whether the collective evidence indicates that short-term exposure to ambient ozone concentrations can affect asthma disease severity. Our causal determination is based on the categorization of the strength of the overall evidence across all realms for or against a causal relationship proposed by the Institute of Medicine (IOM, 2008). The four categories are:

1. *Sufficient*: The evidence is sufficient to conclude that a causal relationship exists.
2. *Equipoise and above*: The evidence is sufficient to conclude that a causal relationship is at least as likely as not, but not sufficient to conclude that a causal relationship exists.
3. *Below equipoise*: The evidence is not sufficient to conclude that a causal relationship is at least as likely as not, or is not sufficient to make a scientifically formed judgment.
4. *Against*: The evidence suggests the lack of a causal relationship.

3. Overview of evidence

3.1. Controlled human exposure studies

We included 34 controlled human exposure studies of individuals with asthma in our systematic review (Described in detail in the

Supplemental materials), of which 30 were Tier I studies. All of the studies used a crossover design, and 28 out of 34 randomized the order of exposures. The number of participants in these studies was typically small, ranging from 5 to 30. The participants exercised in all but four studies, and the exercise protocol varied. Ozone exposure ranged from 1 to 7.6 h in duration, and concentrations ranged from 100 to 400 ppb. These concentrations are well above typical ambient ozone concentrations in the US (*e.g.*, the median daily 8-h maximum ozone concentrations from 2007 to 2009 was 40 ppb). The outcomes assessed in these studies included lung function and airflow parameters, such as forced expiratory volume in 1 s (FEV_1), forced vital capacity (FVC), the ratio of FEV_1 to FVC, forced and peak expiratory flow (FEF and PEF, respectively), airway resistance (*e.g.*, total or specific airway resistance), and respiratory symptoms (*e.g.*, cough and wheezing).

Overall, controlled exposure studies consistently reported temporary respiratory symptoms and decreased lung function following ozone exposure. FEV_1, FVC, other spirometric measures, and respiratory symptoms were not altered to a statistically significant degree after exposure to approximately 100–160 ppb ozone for any duration of exposure, but statistically significant lung function decrements were observed following exposure to higher concentrations 160–400 ppb for 4–8 and 2 h, respectively).

3.2. Epidemiology studies

We included 54 epidemiology studies of asthma exacerbation and severity in our systematic review (Described in detail in the Supplemental materials), of which 39 were Tier I studies. There were two major study designs in the selected epidemiology studies: panel studies and time series/case-crossover studies. Eighteen panel studies evaluated several outcomes of asthma severity in individuals with asthma, including lung function measurements, asthma symptoms and medication use, and occurrence of an acute event, such as an asthma exacerbation or unscheduled doctor or clinic visit for asthma. In contrast, 36 time series and case-crossover studies relied on administrative health data to assess the outcomes of severe asthma exacerbations, such as outpatient/primary care visits, ED visits, HA, and ED admissions.

In general, results from panel studies were largely null for lung function changes and inconsistent with regard to asthma symptoms and medication use. However, studies of asthma-related ED visits and HA, particularly those in Tier I, mostly reported positive, though not always statistically significant, effect estimates for ozone, suggesting that there is an association between short-term exposure to ambient ozone concentrations and asthma severity.

3.3. Animal toxicity studies

We included 12 experimental studies, in which animal models of allergic or obstructive airway or specific lung diseases and allergen sensitization (that mimic the asthma exacerbation process) were employed to evaluate the effects of short-term ozone exposures (Described in detail in the Supplemental materials). The majority of the studies were conducted in rodents (*i.e.*, mice, rats, and guinea pigs), and evaluated AHR, lung resistance, lung compliance and elastance, and other lung function endpoints.

Overall, the most consistent findings were reported for increased AHR following ozone exposures at concentrations of 100 ppb or higher. Results observed for lung resistance, compliance, and other endpoints were less consistent, with some within- and between-study heterogeneity. There was also uncertainty with regard to the relevance of these studies to humans exposed to ambient ozone levels. Most studies were conducted at levels that exceed typical human exposures, with many conducted with exposures an order of magnitude higher than the lowest exposures in the human studies. In addition, interspecies differences in nasal structures, ventilation rates, and body surface area/volume ratios, as well as obligate nose breathing in rodents compared to humans, all limit the relevance of study results to humans (Hatch et al., 2013).

4. Evidence integration

We integrated evidence across realms by considering many of the Bradford Hill aspects, including strength of association, consistency, coherence, biological plausibility, biological gradient (exposure-response), and temporality (Hill, 1965). Although these aspects were developed to interpret epidemiology study results, several apply to other study types as well. We also considered confounding, bias, and the clinical relevance of the effects.

4.1. Strength of association

We assessed the overall magnitude and precision of ozone effects observed in epidemiology studies. To broadly classify the strength of associations, we relied on guidelines suggesting that risk ratios below 1.5 are weak, those above 1.5 are moderate, and those above 3 or 4 are strong (Rosenthal, 1996; Taubes, 1995; Rothman et al., 2008; Boffetta et al., 2008; Fewell et al., 2007). While small effect sizes can have large public health impacts in a large population, associations with small magnitude are more vulnerable to the influences of bias, confounding, or chance than those with large magnitude (Shapiro, 2000). Thus, it is difficult to confidently make causal inferences when the observed associations across a body of observational studies are of small magnitude, because they may be impacted by relatively little bias or confounding that could not be entirely eliminated (Shapiro, 2000). This is a common limitation of environmental epidemiology studies, which often report associations of small magnitude.

Most epidemiology studies of primary care visits, ED visits, and HA for asthma reported relatively small effects; most risk estimates were smaller than 1.5, although there were a few exceptions in both Tier I and Tier II studies. Epidemiology panel studies that evaluated lung function calculated as percentages of predicted lung function metrics typically reported average decrements on the order of 1%. Panel studies that estimated odds or risk of self-reported symptoms following ozone exposures generally calculated risk ratios below 2, with a few outliers in subgroup analyses.

Overall, most epidemiology studies reported associations that were small in magnitude, and many were not statistically significant. Taken together, the magnitude of associations generally observed in this body of evidence does not increase our confidence that observed associations between ozone and asthma severity are causal.

4.2. Consistency and coherence

The results of most controlled exposure studies are consistent in direction across studies, with most lung function changes and all changes in composite symptoms scores in the direction of adversity following ozone exposure. The magnitude of lung function decrements is generally larger, and the results are more consistent in studies conducted with higher ozone concentrations (and/or higher estimated total inhaled doses, which incorporate the effect of exercise duration and intensity).

The effects reported in most animal studies are also consistently in the direction of adversity, *i.e.*, increased AHR and lung resistance, and decreased lung compliance.

Most epidemiology studies of ED visits and HA for asthma, particularly those of higher quality, reported slightly increased risk with elevated short-term ozone exposures. In contrast, panel studies reported mixed results for lung function and asthma symptoms.

Overall, the results within and across realms were generally consistent in the direction of an adverse response. However, controlled exposure experiments conducted at ozone levels similar to ambient levels in the US typically reported null associations, while many

epidemiology studies of asthma-related ED visits and HA reported associations at ambient exposure levels. Potential explanations for the discrepancy include that the small sample sizes in controlled exposure studies are underpowered to detect effects, that chamber conditions do not accurately reflect real-life exposure scenarios, or that the small positive associations in epidemiology studies are spurious or due to bias or confounding.

In summary, we have determined that the degree of consistency within and between studies is moderate. The inconsistency between the ozone concentrations associated with health effects between controlled exposure and epidemiology studies is not easily reconciled.

4.3. Biological gradient

All of the epidemiology studies included in our review evaluated the exposure-response relationship between ozone and asthma severity by modeling ozone exposure as a continuous variable or as quantiles. While many time series and case-crossover studies of ED visits and HA for asthma reported positive, statistically significant gradients, panel studies did not provide support for a biological gradient between ozone exposure and lung function, asthma symptoms and medication use, or occurrence of an acute event such as an asthma exacerbation.

Assessing potential biological gradients within the realms of controlled human exposure and animal studies is more difficult because very few studies evaluated multiple ozone exposures, and studies had varying ozone concentrations, durations, and proportions of time spent exercising. Only five controlled exposure studies (all Tier I) reported results for more than one ozone exposure concentration, and the results of these studies did not clearly reflect a biological gradient. We also assessed the presence of a biological gradient among all of the controlled exposure studies by considering the results of the studies measuring the same outcome metric at different exposure levels (*i.e.*, we calculated a total inhaled dose). Accounting for varying exposure durations and exercise ventilation rates across studies, it appears that the magnitude of FEV_1 decrements increases with increasing inhaled ozone doses across Tier I studies (See Supplemental materials).

Only one animal study tested multiple exposure levels (Larsen et al., 2010), and the greatest effect was observed at the lowest ozone concentration. Because of substantial differences in species, exposure metric, and outcome measure among studies, we did not compare the effects of ozone exposures at varying doses across studies.

Overall, we conclude that the strongest support for a biological gradient comes from epidemiology studies of asthma-related ED visits and HA and controlled human exposure studies that evaluated FEV_1. Evidence from panel studies and the one animal study that evaluated multiple doses, however, does not support a biological gradient.

4.4. Temporality

We evaluated the temporality (*i.e.*, that exposure must precede response) of ozone exposure and asthma severity only in epidemiology studies, because exposure always precedes the response in experimental studies.

As discussed in the Supplemental materials, most epidemiology studies examined multiple lag periods when assessing this association. When the studies used the current-day ambient ozone concentrations as a proxy for ozone exposure, it was not possible to determine whether exposure preceded the health effects that occurred on the same day. However, in analyses with lags of one day or more, it is clear that ozone exposures preceded the health endpoints.

Thus, we conclude that the criterion of temporality is satisfied in human controlled exposure studies and animal experiments and, largely, in epidemiology studies.

4.5. Biological plausibility

Several modes of action (MoAs) have been proposed by which short-term ozone exposure could cause respiratory effects. These MoAs involve the reaction of ozone with components of the extracellular lining fluid of the respiratory tract and cellular membranes, producing secondary oxidation products that can potentially injure the respiratory tract epithelium or cause airway remodeling.

An inflammatory response to asthma triggers, including ozone, has been proposed as a potential mechanism for asthma exacerbation (Sears, 2008; Peden, 1997). At this time, no specific cells have been implicated, and studies show significant heterogeneity and different patterns of inflammation associated with asthma exacerbations. For example, eosinophilic inflammation is typically observed with exacerbations caused by allergen exposure, whereas neutrophilic inflammation has been observed with exacerbations caused by a viral infection (Sears, 2008). Both of these inflammatory responses have been observed in controlled human exposure studies after ozone exposures ranging from 80 to 400 ppb (Peden, 1997). MoA studies have also shown that antioxidants present within airway lining fluid can prevent ozone-mediated cellular responses (Avissar et al., 2000; Ballinger et al., 2005; Cross et al., 1994; Mudway et al., 2001; Samet et al., 2001), and only ozone exposure of a sufficient duration and concentration can overwhelm antioxidant defenses, allowing for an inflammatory response (*e.g.*, Schelegle et al., 2007). Therefore, although ozone can elicit an inflammatory response, this response is likely to occur only at elevated ozone levels.

Another potential MoA that has been proposed by which ozone could elicit a respiratory effect is through a neural mechanism. This possibility is supported by studies that showed that ozone-induced lung function effects can be inhibited by certain drugs, which indicates a vagally mediated influence on the bronchial smooth muscles (reviewed by Prueitt and Goodman, 2016). Irritant and opioid receptors that are potentially stimulated by ozone have also been implicated in effects of ozone on lung function (Peden, 1997).

There is some evidence for other potential MoAs for effects of ozone in asthmatics, such as modification of innate immunity (Hernandez et al., 2012), enhanced responses of purine metabolites in the airways (Esther et al., 2011), and genetic modifiers of oxidative stress (Alexis et al., 2013). Data are limited for these MoAs, particularly at low ozone exposure levels, and the mechanisms by which pollutants, like ozone, alter airway function in asthmatics remain unclear and are in need of further study.

Overall, the specific MoA by which short-term exposure to ozone could affect asthma severity is unknown, but several MoAs have been proposed. Sufficient data are not available, however, to assess whether these mechanisms occur at concentrations that reflect typical US ambient exposures or if they are high-exposure mechanisms. Because we cannot be confident that the proposed mechanisms for respiratory health effects occur at the levels of ozone exposure measured in epidemiology studies, the overall strength of the evidence for causality is diminished.

4.6. Confounding and bias

Confounding and bias, while less of a concern in experimental studies, are well-known limitations of observational epidemiology studies. Air pollution epidemiology studies are considered especially vulnerable, because any true associations between exposure and health are small in magnitude, and therefore more likely to either be spurious or obscured by confounding or bias (Lumley and Sheppard, 2003; Dominici et al., 2003; Goldman et al., 2011; Richiardi et al., 2013).

Several factors have been identified in the research literature as triggers for asthma exacerbation, including many environmental factors such as cold air, dust, pollen, other aeroallergens, and respiratory infections (Barnes, 2008; Sears, 2008; Mayo Clinic, 2014b). Social and

behavioral factors, including stress, poor adherence to asthma maintenance medications, and intense physical activity, can increase the risk of an exacerbation as well (Barnes, 2008; Sears, 2008). The most common trigger of asthma exacerbations in children is viral infections of the upper or lower respiratory tract, which contribute to an estimated 90% of all exacerbations (Barnes, 2008; Sears, 2008). These triggers are often not adequately controlled for in epidemiology studies and may have confounded the observed associations. In addition, all of the controlled exposure studies used a crossover design (*i.e.*, each subject served as their own control) but most did not collect information on all potential contributors to asthma exacerbations that we identified, such as stress and exposure to aeroallergens between individual experiments, within each study period (Described in detail in the Supplemental materials). If any of these factors differed during the study period, it is possible that they influenced the results.

Finally, an important source of bias relevant to *all* realms of research evidence is publication bias. Studies reporting statistically significant results are more likely to be published than those with null findings, resulting in published literature that is not representative of the full body of research data available (Easterbrook et al., 1991; Siddiqi, 2011). Publication bias in studies of ozone exposure and human or animal health effects could lead to misleading results in systematic reviews, with a likely bias in the direction of statistically significant health effects. However, proving that a particular body of research is affected by publication bias is difficult. In a meta-analysis of ozone exposure and respiratory morbidity (Ji et al., 2011), the authors found evidence of potential publication bias in reviewed studies. Based on this finding, we conclude that the literature we reviewed may also be affected by this bias.

In summary, experimental studies are unlikely to have been affected by most types of confounding and bias, though some contributors to asthma exacerbation may have influenced the results from controlled exposure studies. In contrast, all epidemiology studies are susceptible to these factors, and reported associations may be skewed. Without additional data collection and further analyses, it is impossible to know the direction or magnitude of such effects in any one individual study. Publication bias may have biased studies in all the realms in the direction of positive associations between ozone and asthma severity. Overall, the strength of the evidence for causation is reduced by the likely presence of confounding and biases.

4.7. Clinical relevance

We considered whether the various outcomes evaluated in reviewed studies are clinically relevant indicators of disease severity.

Increased use of urgent healthcare resources, including ED visits and HA, is clearly clinically important and relevant to our research question (Reddel et al., 2009). Many time series and case-crossover studies reported that population-wide rates of ED visits and HA with a diagnosis code for asthma were higher in the days following elevated ambient ozone levels.

Some panel studies also assessed outcomes directly relevant to asthma exacerbations, albeit less severe, including self-reported asthma attacks, increased use of "quick relief" asthma medication, missed school attributable to asthma, or unscheduled physician visits for asthma. In general, results for these associations were less consistent.

For panel studies of lung function and self-reported symptoms, and controlled exposure studies of lung function and asthma symptoms, the relevance of the findings to asthma severity is more uncertain. Temporary lung function decrements and increases in respiratory symptoms may resolve quickly with or without treatment and are not necessarily associated with worsening disease status or loss of asthma control in the short or long term (Reddel et al., 2009).

Finally, the limited utility and relevance of animal studies of asthma should be taken into consideration. Animal models of asthma are artificial because typical laboratory animals (*e.g.*, rodents) do not spontaneously develop asthma (Szelenyi, 2000; Nials and Uddin, 2008). In addition, there are species- and strain-specific physiological differences that limit the extrapolation from experimental animals to human. Further, study-to-study methodological differences, including variations in sensitization and challenge protocols, and drug form and delivery systems (*e.g.*, intranasal *versus* intratracheal, nebulized solutions *versus* dry powders), make it difficult to compare results across studies (Holmes et al., 2011).

In summary, the uncertainty regarding the relevance of measures of lung function to long-term increases in asthma severity limits the use of these measures in determining the causal relationship between ozone and asthma severity. Positive associations between ambient ozone and population-wide rates of ED visits and HA for asthma observed in a large number of epidemiology studies, however, provide evidence of clinically relevant effects for asthma exacerbations.

4.8. Causal determination

Using principles of a hypothesis-based WoE approach (Rhomberg et al., 2010), we weighed the plausibility that short-term ozone exposure at typical ambient concentrations in the US is causally associated with asthma severity against the likelihood that observed positive associations in the research literature are accounted for by alternative explanations.

Epidemiology studies that reported increased ED visits and HA for asthma following elevated ozone levels provide the strongest support for the hypothesis that short-term exposure to ambient ozone concentrations affects asthma severity, including causing an increase in asthma exacerbation events. This evidence is strengthened by the consistency across studies, the presence of a biological gradient, and the clinical relevance of the outcomes, but is undermined by the possible presence of confounding and bias. Controlled human exposure studies consistently reported reduced lung function following ozone exposures in a concentration-dependent manner, and these also support a potential causal relationship, but this experimental evidence is undermined by uncertainties arising from ozone exposures much higher than ambient concentrations and the clinical relevance of lung function outcomes to disease severity. Evidence from animal toxicity studies does not provide support for a causal relationship, but it does not refute it either because its relevance to humans is unclear.

We next considered the possibility that short-term exposure to ambient ozone concentrations does not affect asthma severity, and we identified several plausible alternative explanations for the positive associations in the body of literature. The relatively small associations observed in controlled human exposure and epidemiology studies could be attributable to bias, chance, or confounding. As discussed above, confounding by other triggers for asthma exacerbation (*e.g.*, aeroallergens or respiratory infections), multiple testing, and publication bias could explain the small positive associations between ozone and asthma-related ED visits and HA, asthma symptoms, and lung function observed in multiple epidemiology studies. Similarly, despite the experimental design of controlled human exposure studies, failure to account for certain asthma triggers could contribute to observed decrements in lung function. In addition, temporary lung function decrements are not necessarily associated with symptoms or lasting adverse effects. Finally, the ozone concentrations that were consistently associated with effects in controlled exposure studies are considerably higher than typical ambient concentrations in the US. Therefore, the observation of temporarily decreased lung function following high ozone exposures does not contradict the hypothesis that no causal relationship exists between asthma severity and ozone exposure at ambient concentrations.

In summary, we found that there is substantial uncertainty associated with each of the two competing hypotheses: 1) there is a casual relationship between short-term ozone at typical ambient concentrations in the US and asthma severity; and 2) the observed association is

not causal. We therefore determined that there is at least an *equal* likelihood that either explanation is true, *i.e.*, the strength of the evidence for a causal relationship between short-term exposure to ambient ozone concentrations and asthma severity is "***equipoise and above***."

5. Conclusions

During the most recent review of the ozone NAAQS, US EPA concluded that short-term ozone exposure causes respiratory morbidity and that individuals with asthma constitute a group susceptible to ozone. These conclusions were based largely on observed associations between short-term ozone exposure and various outcomes related to asthma severity, including ED visits and HA for asthma, and changes in lung function and reported respiratory symptoms in individuals with asthma (US EPA, 2013).

However, while the totality of the evidence is suggestive of an association between short-term exposure to ambient ozone concentrations and asthma severity, it is not sufficiently strong to infer a causal relationship, and several plausible alternative explanations for positive findings cannot be ruled out with confidence. We conclude that the evidence is sufficient to conclude that a causal relationship is at least as likely as not, but not sufficient to conclude that a causal relationship exists. The substantial uncertainty in the body of evidence should be taken into consideration when this evidence is used for policymaking.

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Declaration of interest

The authors are current (JEG, KZ, HNL, RLP, IM, SPS) and former (CTL, SNS) employees of Gradient, an independent environmental and risk science consulting firm, in Cambridge, Massachusetts. The work reported in this paper was conducted by the authors during the normal course of their employment by Gradient, and the authors have the sole responsibility for the writing, content, and conclusions in this article. Dr. Goodman, Dr. Zu, Dr. Loftus, Ms. Lynch, Dr. Prueitt, and Dr. Sax have provided written and/or oral comments to US EPA and/or the US EPA Clean Air Scientific Advisory Committee (CASAC) on various NAAQS documents of criteria air pollutants, with funding provided by several trade organizations. Dr. Goodman, Dr. Zu, Dr. Loftus, Ms. Lynch, Dr. Prueitt, and Dr. Sax have also authored review articles and/or conducted original research on the health effects associated with ambient air pollutants.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.envres.2017.10.018.

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